

Quantitative evaluation of the paralytic activity of an ENF peptide in *Bombyx mori*

WANG Fei*, DONG Shi-Feng, SONG Liang, HU Jie, XIA Qing-You

(State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400716, China)

Abstract: 【Aim】 A Glu-Asn-Phe (ENF) peptide, paralytic peptide (PP) identified in several lepidopteran hemolymph induces rapid and rigid paralysis defined by a tonic contraction when injected into larvae. This study aims to determine the optimal amount and maximum contraction that PP induces in live larvae of the silkworm, *Bombyx mori*, and to examine the possible change of other physiological index, such as pulse velocity and ion concentration which might accompany the contraction. 【Methods】 The intensity of body contraction and pulse velocity were monitored after injection of various amounts of PP into the body of the 5th instar larvae. And the ion concentrations in hemolymph, fat body and digestive tube were measured by atomic absorption spectroscopy. 【Results】 PP at the concentration of 50 ng/g animal triggered the most potent contraction with no lethal effect. The maximum body contraction was seen between 4 and 5 min after injection. Digestive tube distortion was found to accompany body wall contraction, and pulse velocity decreased when the body reached the maximum contraction. In addition, extracellular Ca^{2+} was required for the contraction and PP also stimulated a sharp decrease then a slow recovery of Cl^- concentration in hemolymph. 【Conclusion】 The paralytic activity of PP not only affects the body wall, but also digestive tube and dorsal vessel of silkworm larvae, and is associated with disruption of Cl^- homeostasis. Our results will provide reference for studying the physiological role of PP in an animal model.

Key words: *Bombyx mori*; paralytic peptide; contraction; ion concentration; pulse velocity

1 INTRODUCTION

A group of bio-active peptides, which contain the same signature Glu-Asn-Phe (ENF) sequence at the N terminus, have been identified in Lepidoptera decades ago (Hayakawa, 1990; Skinner *et al.*, 1991; Clark *et al.*, 1997; Furuya *et al.*, 1999; Ha *et al.*, 1999). These ENF peptides, typically 23 or 25 amino acids long, are generated from the C-terminal region of longer precursors, which are mainly synthesized in fat body and released into the hemolymph (Matsumoto *et al.*, 2012). Once triggered by immune challenges, such as bacterial infection, wasp parasitization or injury, these precursors are processed into the active peptides by certain serine protease. ENF peptides were individually named after the physiological functions, which they were initially considered to play. For instance, the peptide purified from *Pseudaletia separate* was designated growth blocking peptide (GBP) since it retarded larval development (Hayakawa, 1990). And the peptide isolated from *Pseudoplusia includens* was referred to as plasmatocyte spreading peptide (PSP) because it

induced plasmatocytes to spread (Clark *et al.*, 1997). Paralytic peptide (PP) which caused rapid paralysis was identified later in *Bombyx mori*, *Manduca sexta*, *Spodoptera exigua* and *Heliothis virescens* (Skinner *et al.*, 1991; Ha *et al.*, 1999). Although their functions sound different as indicated by their names, their sequences share high homology with each other, suggesting they may exert similar biological activities. In fact, GBP and PP were reported to be able to stimulate the spreading of plasmatocytes (Wang *et al.*, 1999; Aizawa, *et al.*, 2002; Nakahara *et al.*, 2003), and PSP could delay the onset of metamorphosis as well (Strand *et al.*, 2000).

The amount of ENF peptide fluctuates during development and varies among species, probably due to the different methods used for quantitation. For instance, in the 4th instar silkworm larvae, the amount of PP is approximately 200 ng/individual estimated from the spectral absorption (Ha *et al.*, 1999), whereas in the 5th instar larvae of *P. includens* PSP concentration is 180 ng/mL determined by a quantitative immunoassay using a PSP-specific antibody (Clark *et al.*, 1997, 2005). Interestingly, bacterial and viral infection, wasp

基金项目: 国家自然科学基金项目(31672495); 重庆市基础与前沿研究计划项目(CSTC2014JCYJA80010)

作者简介: 王菲, 女, 1978年7月生, 湖北武汉人, 博士, 副教授, 研究方向为家蚕分子免疫学, E-mail: fwangswu@gmail.com

* 通讯作者 Corresponding author, E-mail: fwangswu@gmail.com

收稿日期 Received: 2017-07-18; 接受日期 Accepted: 2017-08-27

parasitization, low temperature and hormone treatment all elevated the expression level of ENF peptides, suggesting they may be playing multiple biological activities (Ohnishi *et al.*, 1995; Hayakawa *et al.*, 1998; Kamimura *et al.*, 2001; Eleftherianos *et al.*, 2009; Ishii *et al.*, 2010a).

Studies on the ENF-regulated immune response revealed several signaling molecules and cascades are involved (Ishii *et al.*, 2010b, 2013; Oda *et al.*, 2010; Song *et al.*, 2015). However, some other physiological changes, such as paralysis or developmental retardation, are not well addressed, partially owing to the lack of measurement of accompanying physiological reactions. For instance, muscle contraction seems to be the only definition of “paralysis”, although actually the body rigidity is usually accompanied by a sudden change of other physiological index, such as cardiac rhythm. This study was undertaken in an attempt to evaluate the biological activities of PP in broader aspects in live animals.

2 MATERIALS AND METHODS

2.1 Insect

Silkworm (*B. mori*) Dazao P50 strain and *oc* strain (oily silkworm) were originally obtained from Silkworm Genetic Resource Supply in Southwest University. Larvae were reared on fresh mulberry leaves at 25°C and relative humidity of 80%. The day-2 2nd instar larvae weighing 900 – 1 000 mg were used for experiments.

2.2 Body contraction assay

PP was chemically synthesized as described previously (Song *et al.*, 2015) and dissolved in phosphate-buffered saline (20 mmol/L PBS, 0.0162 mol/L Na_2HPO_4 , 0.0038 mol/L NaH_2PO_4 , pH 7.4). The day-2 5th instar larva of silkworm was injected with 10, 20, 50 and 100 ng PP, respectively, through the second last stoma in abdomen with a fine needle. In some experiments, 10 μL 0.5 mol/L EGTA or EDTA was injected into each larva 15 min before PP injection.

The intensity of body contraction was expressed as the contraction ratio, calculated by measuring the body length of each larva before (x cm) and after (y cm) the injection using the formula $(x - y)/x$. To facilitate the measurement of body length, unparalyzed larvae were placed on ice to reduce their locomotor activity temporarily. At least six larvae were used in each treatment and at least three separate experiments were performed.

2.3 Ion concentration analysis

Hemolymph was collected from incisions at the

larva leg. Then fat body was collected from dissected larvae and blotted gently to remove residual hemolymph. After removing peritrophic membrane and food content, digestive tube was rinsed quickly, and then blotted to remove extra water. All tissue samples were placed into pre-weighted 1.5 mL tubes and dried at 70°C for 48 h. Tissue water content was determined gravimetrically from the mass before and after being dried. Two hundred μL nitric acid was added to the dried samples and incubated for 24 h at room temperature. Total tissue Na^+ , K^+ , Ca^{2+} and Cl^- concentrations were determined using atomic absorption spectroscopy (Z-5000, Hitachi, Japan).

2.4 Pulse velocity recording

Four min after PP injection (50 ng per larva), pulse velocity was timed by counting the systolic contraction of dorsal vessel which was visible through the translucent larva skin of *oc* strain.

2.5 Statistical analysis

Data were presented as the mean \pm SD ($n \geq 3$). Statistical significant differences were determined by Student's *t*-test.

3 RESULTS

3.1 Injection of PP induces sustained contraction

As shown in Fig. 1 (A), injection of PP caused a rigid paralysis of silkworm larvae as if they were frozen. Contraction of the body was apparent, the body segments shortening and the thoracic region swelling. Severe intestinal distortion with food (debris of mulberry leaves) accumulated in the foregut and anterior region of midgut was clearly seen after dissection. Regurgitation or evacuation was also observed in some larvae, and these larvae were usually unable to recover and died afterwards. To evaluate the contraction-inducing effect of PP, we first measured the time required to reach maximum contraction as designated by contraction ratio after injection of PP at different dosages. The maximum contraction was seen between 4 and 5 min after injection (Fig. 1; B). During this time window, the body length of silkworm larvae was shrunk by 15% to 22%, and higher dosage resulted in faster and greater contraction (Fig. 1; B, C). However, since almost half of larvae under the treatment of 100 ng per larvae could not survive after injection, 50 ng per larva was chosen as the dose of injection in further experiments. The larvae gradually recovered after 1 h, and fully returned to physiological activity in hours.

3.2 EGTA reduces contraction induced by PP

Considering the body rigidity may be a result of muscle contraction which usually involves increasing

of free intracellular Ca^{2+} , we tested whether extracellular calcium influx is required for the contraction. EDTA or EGTA was injected into the body 15 min before PP treatment. As shown in Fig. 2, EGTA inhibited the contraction-inducing effect of PP since no paralysis was observed in silkworm larvae pre-injected with EGTA. On the contrary, EDTA only partially attenuated the effect, since body contraction was still observed, merely to a

lesser extent. Compared with EDTA, EGTA is more preferable in chelating Ca^{2+} , therefore it can keep more extracellular calcium from entering into the cell to trigger the contraction of cytoskeleton. The inhibition of PP-induced contraction by EGTA suggests that PP treatment may mobilize certain ligand-gated calcium channel on cytoplasmic membrane.

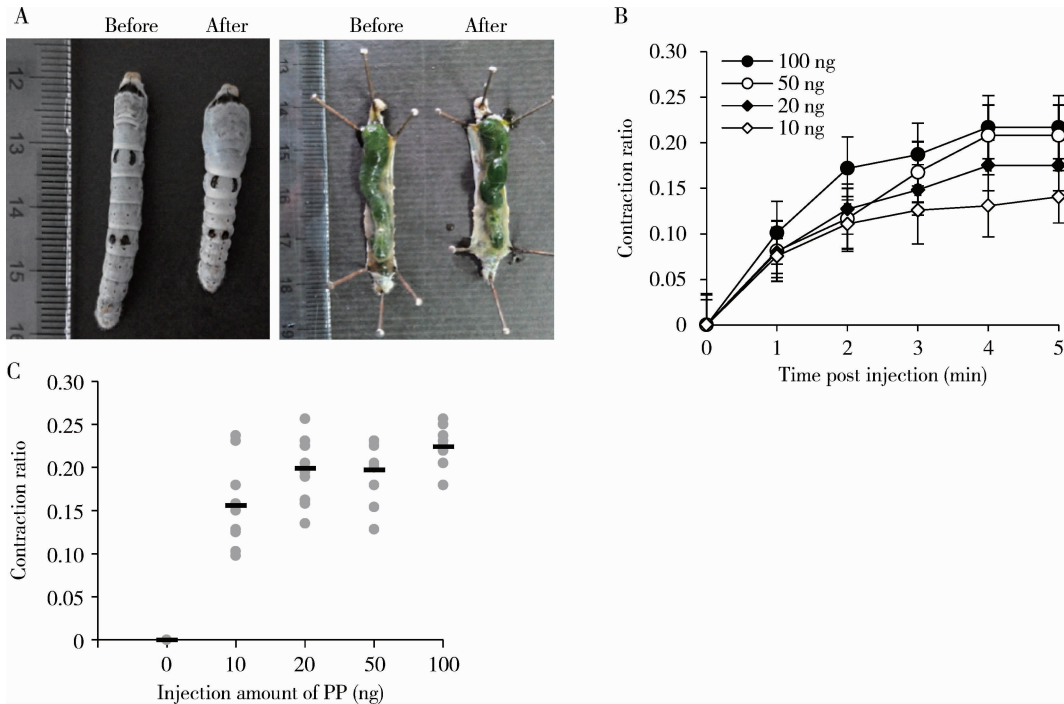


Fig. 1 Body contraction assay of *Bombyx mori* larvae after injection of paralytic peptide (PP)

A: Body (left) and digestive tube (right) of silkworm larvae before and after PP injection. B: Body contraction ratio measured at different time after PP injection. Each point represents mean \pm SD of at least 10 individuals. C: Body contraction ratios caused by different amounts of PP measured at 5 min after injection. Grey round spots represent contraction ratios of individual larvae; short black lines represent median contraction ratios.

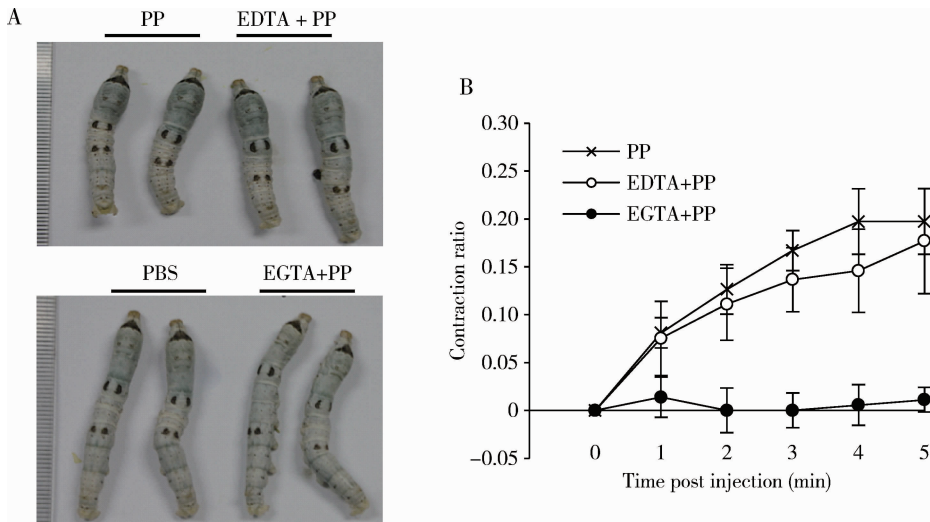


Fig. 2 Body contraction of *Bombyx mori* larvae pre-injected with EGTA or EDTA

Silkworm larvae injected with indicated reagents (A), and body contraction ratio of larvae pretreated with EDTA or EGTA measured at different time after PP injection (B). Each point represents mean \pm SD of at least 6 individuals.

3.3 Cl⁻ concentration in hemolymph is decreased after PP injection

Beside calcium, we were also interested in whether any other intracellular or extracellular ions were affected by PP treatment that may account for this sustained paralysis. Therefore, we tracked the concentrations of Na⁺, K⁺, Ca²⁺ and Cl⁻ in hemolymph, fat body and gut after PP treatment. No distinct fluctuation of ion concentration was detected except Cl⁻ in hemolymph, which showed a fast decrease to about 60% within 5 min after PP injection, and then gradually restored afterwards (Fig. 3).

3.4 Pulse velocity is decreased after PP injection

The dorsal vessel was clearly seen in normal silkworm larvae and pulse velocity was easily to be measured by counting the vessel pulse per min. However, body contraction induced by PP caused wrinkles on epidermis, making it inconvenient to visualize the vessel or inaccurate for counting. Therefore, we used the oily silkworm larvae, which had semi-transparent epidermis and displayed similar

paralysis phenotype after PP injection, to examine vessel contraction (Fig. 4: A). Pulse velocity in both Dazao and *oc* strain decreased significantly after PP injection. More than 40% decrease was even noticed in *oc* strain (Fig. 4: B).

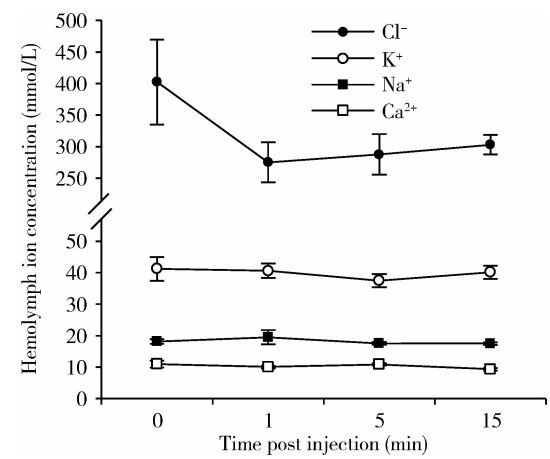


Fig. 3 Hemolymph ion concentration at indicated time after PP injection

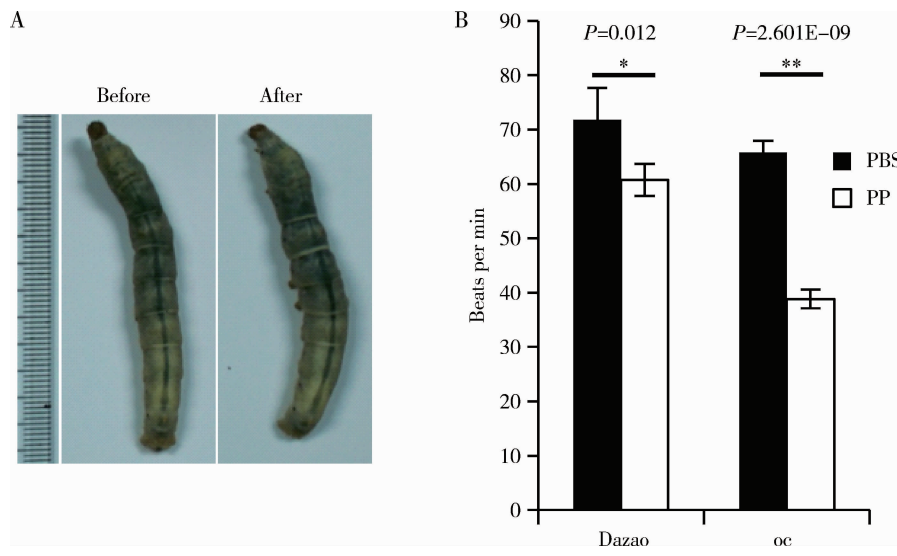


Fig. 4 Pulse velocity of *Bombyx mori* larvae after PP injection

A: Body contraction of *oc* strain before and after PP injection; B: Pulse velocity of Dazao and *oc* strains counted at 4 min after PP injection as compared to PBS injection. *P* values determined by Student's *t*-test are indicated between PP and PBS treatment. Asterisks denote significant difference (* *P* < 0.05, ** *P* < 0.01).

4 DISCUSSION AND CONCLUSION

The onset of paralysis defined by body contraction and rigidity after injection of hemolymph drawn from one lepidopteran larva to another triggered research interest that led to the discovery of PP in various species (Skinner *et al.*, 1991). PP was initially considered to be a neuropeptide, but later studies found that paralysis cannot be inhibited by receptor antagonist at the nerve-muscle junction, suggesting that PP may directly control muscle cell

activity without evoking nerve cells (Sekimizu *et al.*, 2005; Ishii *et al.*, 2008). Interestingly, injection of yeast β -glucans or bacterial peptidoglycans into silkworm larvae also induced paralysis which has been proved to be mediated by PP since those fungal and bacterial wall components promoted the cleavage of PP precursor to active peptide in hemolymph (Fujiyuki *et al.*, 2012). Based on this observation, a screening method has been proposed by using this easy-to-measure muscle contraction assay to evaluate the immune stimulating activity of natural substances (Ishii *et al.*, 2015).

The amount of PP required for the maximum body contraction tested in decapitated silkworm (about 40 ng/g animal) (Ishii *et al.*, 2008) is similar to the amount of PP tested in live larvae in our study. However, the maximum contraction ratio of decapitated silkworm with peritrophic membranes removed (more than 40%) was higher than that of live larvae (about 20%), suggesting that some organs other than body wall were affected. After dissection, we observed the twisting of digestive tube that might be the result of abnormal contraction of gut visceral muscle. Then the loss of gut motility, at least temporarily, could cause slower weight gain that was seen in ENF peptide-injected larvae.

The inhibition of PP-induced response by cation chelator EGTA revealed that the effects of PP on silkworm muscle was dependent on extracellular calcium, whose concentration was usually more than 10 000-fold higher than intracellular calcium. The sharp decrease of hemolymph Cl^- might be the result of opening of calcium-activated chloride channels on muscles (Eggermont, 2004). Additionally, the sustained low concentration of Cl^- in hemolymph afterwards reflected a slow recovery of homeostasis. Whether the calcium-activated chloride currents contribute to the magnitude and duration of contraction needs further electrophysiological studies.

Previous study on another ENF peptide isolated from *Spodoptera eridania* claimed that it has excitatory effects on semi-isolated hearts from *M. sexta* larvae (Furuya *et al.*, 1999). Although we did notice an increase of pulse velocity seconds after PP injection to the whole body of silkworm, injection of saline or H_2O alone also resulted in the similar increase in pulse rate. So we suspected that the physical stress caused by injection itself may mask the effect of PP when pulse velocity was measured within a short time right after injection. Unexpectedly, we found a decrease of pulse velocity when muscle reached the maximum contraction and this lower pulse rate sustained for a few minutes. We thought that the decrease in pulse rate might be related to the retardance in development that has been reported in ENF peptide-treated larvae. The physiological significance between the excitatory effects of PP on larval body muscles and inhibitory effect on dorsal vessel is yet to be determined.

To date, no specific receptor of PP or any other ENF peptide has been identified. Although studies on the structure of ENF peptides revealed the resemblance of their tertiary structures to the C-terminal subdomain of mammalian epidermal growth

factor (EGF) (Volkman *et al.*, 1999; Aizawa *et al.*, 2002; Miura *et al.*, 2002), there was no direct evidence that PP binds to epidermal growth factor receptor (EGFR) (Ohnishi *et al.*, 2001). The loss of immunostimulatory effect of PP in the presence of EGFR inhibitors demonstrated that PP activates immune response through EGFR pathway (Song *et al.*, 2015). However, those inhibitors did not affect the contraction-inducing function of PP (data not shown). We previously reported that PP treatment up-regulated the phosphorylation level of several protein kinases, including G protein-coupled receptor kinase 2 (GRK2) (Song *et al.*, 2017), which in mouse attenuates contractility of cardiomyocytes (Fu *et al.*, 2015). Further investigation is required to understand whether PP regulates vessel pulse through activating GRK2 in silkworm.

In summary, by quantitative study we determined the optimal amount and maximum contraction that PP induces in live animals. We also found the paralytic activity not only affects the body wall, but also the intestine and dorsal vessel of silkworm larvae, and is associated with disruption of Cl^- homeostasis. Our results provide reference for studying the physiological role of PP in an animal model.

References

- Aizawa T, Hayakawa Y, Nitta K, Kawano K, 2002. Structure and activity of insect cytokine GBP which stimulates the EGF receptor. *Mol. Cells*, 14: 1–8.
- Clark KD, Kim Y, Strand MR, 2005. Plasmacyte sensitivity to plasmacyte spreading peptide (PSP) fluctuates with the larval molting cycle. *J. Insect Physiol.*, 51: 587–596.
- Clark KD, Pech LL, Strand MR, 1997. Isolation and identification of a plasmacyte-spreading peptide from the hemolymph of the lepidopteran insect *Pseudoplusia includens*. *J. Biol. Chem.*, 272: 23440–23447.
- Eggermont J, 2004. Calcium-activated chloride channels: (un)known, (un)loved? *Proc. Am. Thorac. Soc.*, 1: 22–27.
- Eleftherianos I, Xu M, Yadi H, French-Constant RH, Reynolds SE, 2009. Plasmacyte-spreading peptide (PSP) plays a central role in insect cellular immune defenses against bacterial infection. *J. Exp. Biol.*, 212: 1840–1848.
- Fu Q, Xu B, Parikh D, Cervantes D, Xiang YK, 2015. Insulin induces IRS2-dependent and GRK2-mediated $\beta(2)$ AR internalization to attenuate β AR signaling in cardiomyocytes. *Cell. Signal.*, 27: 707–715.
- Fujiyuki T, Hamamoto H, Ishii K, Urai M, Kataoka K, Takeda T, Shibata S, Sekimizu K, 2012. Evaluation of innate immune stimulating activity of polysaccharides using a silkworm (*Bombyx mori*) muscle contraction assay. *Drug Discov. Ther.*, 6: 88–93.
- Furuya K, Hackett M, Cirelli MA, Schegg KM, Wang HL, Shabanowitz J, Hunt DF, Schooley DA, 1999. A cardioactive peptide from the southern armyworm, *Spodoptera eridania*. *Peptides*, 20: 53–61.
- Ha SD, Nagata S, Suzuki A, Kataoka H, 1999. Isolation and structure determination of a paralytic peptide from the hemolymph of the silkworm, *Bombyx mori*. *Peptides*, 20: 561–568.
- Hayakawa Y, 1990. Juvenile-hormone esterase-activity repressive factor in the plasma of parasitized insect larvae. *J. Biol. Chem.*, 265:

- 10813 – 10816.
- Hayakawa Y, Ohnishi A, Endo Y, 1998. Mechanism of parasitism-induced elevation of haemolymph growth-blocking peptide levels in host insect larvae (*Pseudaletia separata*). *J. Insect Physiol.*, 44: 859 – 866.
- Ishii K, Adachi T, Hamamoto H, Oonishi T, Kamimura M, Imamura K, Sekimizu K, 2013. Insect cytokine paralytic peptide activates innate immunity via nitric oxide production in the silkworm *Bombyx mori*. *Dev. Comp. Immunol.*, 39: 147 – 153.
- Ishii K, Hamamoto H, Imamura K, Adachi T, Shoji M, Nakayama K, Sekimizu K, 2010a. *Porphyromonas gingivalis* peptidoglycans induce excessive activation of the innate immune system in silkworm larvae. *J. Biol. Chem.*, 285: 33338 – 33347.
- Ishii K, Hamamoto H, Kamimura M, Nakamura Y, Noda H, Imamura K, Mita K, Sekimizu K, 2010b. Insect cytokine paralytic peptide (PP) induces cellular and humoral immune responses in the silkworm *Bombyx mori*. *J. Biol. Chem.*, 285: 28635 – 28642.
- Ishii K, Hamamoto H, Kamimura M, Sekimizu K, 2008. Activation of the silkworm cytokine by bacterial and fungal cell wall components via a reactive oxygen species-triggered mechanism. *J. Biol. Chem.*, 283: 2185 – 2191.
- Ishii K, Hamamoto H, Sekimizu K, 2015. Studies of host-pathogen interactions and immune-related drug development using the silkworm: interdisciplinary immunology, microbiology, and pharmacology studies. *Drug Discov. Ther.*, 9: 238 – 246.
- Kamimura M, Nakahara Y, Kanamori Y, Tsuzuki S, Hayakawa Y, Kiuchi M, 2001. Molecular cloning of silkworm paralytic peptide and its developmental regulation. *Biochem. Biophys. Res. Commun.*, 286: 67 – 73.
- Matsumoto H, Tsuzuki S, Date-Ito A, Ohnishi A, Hayakawa Y, 2012. Characteristics common to a cytokine family spanning five orders of insects. *Insect Biochem. Mol. Biol.*, 42: 446 – 454.
- Miura K, Kamimura M, Aizawa T, Kiuchi M, Hayakawa Y, Mizuguchi M, Kawano K, 2002. Solution structure of paralytic peptide of silkworm, *Bombyx mori*. *Peptides*, 23: 2111 – 2116.
- Nakahara Y, Kanamori Y, Kiuchi M, Kamimura M, 2003. Effects of silkworm paralytic peptide on in vitro hematopoiesis and plasmacytocyte spreading. *Arch. Insect Biochem. Physiol.*, 52: 163 – 174.
- Oda Y, Matsumoto H, Kurakake M, Ochiai M, Ohnishi A, Hayakawa Y, 2010. Adaptor protein is essential for insect cytokine signaling in hemocytes. *Proc. Natl. Acad. Sci. USA*, 107: 15862 – 15867.
- Ohnishi A, Hayakawa Y, Matsuda Y, Kwon KW, Takahashi TA, Sekiguchi S, 1995. Growth-blocking peptide titer during larval development of parasitized and cold-stressed armyworm. *Insect Biochem. Mol. Biol.*, 25: 1121 – 1127.
- Ohnishi A, Oda Y, Hayakawa Y, 2001. Characterization of receptors of insect cytokine, growth-blocking peptide, in human keratinocyte and insect S9 cells. *J. Biol. Chem.*, 276: 37974 – 37979.
- Sekimizu K, Larranaga J, Hamamoto H, Sekine M, Furuchi T, Katane M, Homma H, Matsuki N, 2005. D-Glutamic acid-induced muscle contraction in the silkworm, *Bombyx mori*. *J. Biochem.*, 137: 199 – 203.
- Skinner WS, Dennis PA, Li JP, Summerfelt RM, Carney RL, Quistad GB, 1991. Isolation and identification of paralytic peptides from hemolymph of the lepidopteran insects *Manduca sexta*, *Spodoptera exigua*, and *Heliothis virescens*. *J. Biol. Chem.*, 266: 12873 – 12877.
- Song L, Wang F, Dong S, Hu C, Hua X, Xia Q, 2015. Paralytic peptide activates insect humoral immune response via epidermal growth factor receptor. *Peptides*, 71: 20 – 27.
- Song L, Wang F, Dong Z, Hua X, Xia Q, 2017. Label-free quantitative phosphoproteomic profiling of cellular response induced by an insect cytokine paralytic peptide. *J. Proteomics*, 154: 49 – 58.
- Strand MR, Hayakawa Y, Clark KD, 2000. Plasmacytocyte spreading peptide (PSP1) and growth blocking peptide (GBP) are multifunctional homologs. *J. Insect Physiol.*, 46: 817 – 824.
- Volkman BF, Anderson ME, Clark KD, Hayakawa Y, Strand MR, Markley JL, 1999. Structure of the insect cytokine peptide plasmacytocyte-spreading peptide 1 from *Pseudoplusia includens*. *J. Biol. Chem.*, 274: 4493 – 4496.
- Wang Y, Jiang HB, Kanost MR, 1999. Biological activity of *Manduca sexta* paralytic and plasmacytocyte spreading peptide and primary structure of its hemolymph precursor. *Insect Biochem. Mol. Biol.*, 29: 1075 – 1086.

家蚕 ENF 肽致麻痹活性的定量评估

王菲*, 董世峰, 宋亮, 胡杰, 夏庆友

(西南大学家蚕基因组生物学国家重点实验室, 重庆 400716)

摘要:【目的】麻痹肽(paralytic peptide, PP)是一种在鳞翅目昆虫血淋巴中鉴定到的 Glu-Asn-Phe (ENF)肽,将其注入幼虫体内会引发快速僵化的紧张性收缩。本研究旨在确定 PP 引起家蚕 *Bombyx mori* 幼虫活体收缩的最适剂量和最大收缩幅度,并检测诸如背血管搏动速率和离子浓度等可能伴随收缩现象的其他生理指标的变化。【方法】向家蚕 5 龄幼虫注射不同剂量的 PP 后,监测其体长收缩幅度和背血管搏动速率,并用原子吸收光谱测定血淋巴、脂肪体和肠道的离子浓度。【结果】浓度为 50 ng/g 动物时,PP 引发最有力收缩且不会导致家蚕死亡。最大收缩出现在注射后 4~5 min 内,同时肠道出现不正常扭曲以及背血管脉动速率下降。此外,细胞外 Ca^{2+} 是收缩所必需的,PP 刺激还导致血淋巴中 Cl^- 浓度急剧下降而后缓慢恢复。【结论】PP 的致麻痹活动不仅引起身体收缩,还会影响家蚕幼虫的肠道和背血管的脉动,而且破坏 Cl^- 的体内平衡。研究结果为在动物模型中研究 PP 的生理功能提供参考。

关键词: 家蚕; 麻痹肽; 收缩; 离子浓度; 脉动速率

中图分类号: Q966 **文献标识码:** A **文章编号:** 0454-6296(2017)10-1114-06

(责任编辑: 赵利辉)